Immunology 2000 **99** 367–374

Effective formation of major histocompatibility complex class II–peptide complexes from endogenous antigen by thyroid epithelial cells

R. MAILE,* K. A. ELSEGOOD,* T. C. HARDING,* J. B. UNEY,* C. E. H. STEWART,† G. BANTING‡ & C. M. DAYAN* **Division of Medicine, University of Bristol, Bristol Royal Infirmary, Bristol, UK, †University Department of Surgery, Bristol Royal Infirmary, Bristol, UK, and ‡Department of Biochemistry, University of Bristol, School of Biomedical Sciences, Bristol, UK*

SUMMARY

In autoimmune thyroid disease, thyroid epithelial cells (TEC) express major histocompatibility complex (MHC) class II molecules, potentially enabling them to present thyroid self-antigens to CD4-positive T cells. However, despite this, TEC may fail to present endogenous antigen as a result of limited processing or MHC class II loading capacity, or inadequate MHC class II levels. We addressed these issues using the cloned rat TEC line, Fischer rat thyroid cell line (FRTL5), which was transfected using an adenoviral expression vector that expressed ovalbumin (OVA) as an integral membrane protein. OVA-expressing FRTL5 cells very efficiently activated a panel of OVA-specific, class II-restricted T-cell hybridomas. This response was dependent on induction of MHC class II molecules by interferon-γ (IFN-γ) and was blocked by anti-MHC class II antibodies. Poor responses were seen to exogenously added OVA or OVA peptides. These results provide the most direct evidence to date that TEC can form MHC class II–peptide complexes derived from self-antigen in sufficient quantities to activate T cells.

INTRODUCTION

Autoimmune thyroiditis is the most common autoimmune disease seen in humans.[1] Major histocompatibility complex (MHC) class II molecules are not present on thyroid epithelial cells (TEC) from normal thyroid tissue, but class II expression on TEC is frequently seen in autoimmune thyroid disease, almost certainly in response to cytokine (predominantly interferon-γ [IFN-γ]) secretion by the lymphocytic infiltrate.[2,3] If endogenous self-antigens present within the TEC can gain access to these MHC class II molecules, this raises the possibility that TEC can directly engage the antigen receptor of self-reactive $CD4^+$ T cells. Such an interaction may then activate these T cells, fuelling the autoimmune process, as originally proposed by Bottazzo *et al.*[4] Alternatively, depending on the costimulatory ability of the TEC, the interaction may result in T-cell anergy or apoptosis, representing a protective mechanism to down-regulate autoimmunity.[5] Either result is likely to have significant effects on the autoimmune process. Hence it is important to establish whether endogenous antigens can indeed gain access to the MHC class II pathway in TEC.

It has previously been shown that TEC derived from autoimmune glands can present viral peptides to T cells and restimulate cloned thyroid-specific T cells derived from the same gland;[6,7] however, contamination by dendritic cells in these studies cannot be excluded. Lombardi *et al.* found that human TEC were able to stimulate T cells that were not dependent on B7 for costimulation and were able to 'anergize' cells that were B7 dependent,[8] but viral peptides were again used as a source of antigen. Others have found human TEC to have only weak antigen-presenting function,[9,10] but the ability of TEC to present endogenous proteins was not examined. In rodent studies, some workers,[11,12] but not others,[13–15] have been able to show antigen presentation by TEC, but again the role of endogenous antigen could only be assessed indirectly.[16]

To address, more directly, the issue of access to the MHC class II pathway in TEC by endogenous antigen, we developed a system in which the cDNA encoding a test antigen was introduced into a thyroid cell line, and cloned T-cell hybridomas (which are not subject to the variable additional costimulatory

Received 13 July 1999; revised 11 October 1999; accepted 13 October 1999.

Abbreviations: c.p.m., counts per minute; FCS, fetal calf serum; FRTL5, Fischer rat thyroid cell line; HAT, hypoxanthine/aminopterin/thymidine; HMG, horse myoglobin; IFN-γ, interferon-γ; LNC, lymph node cells; OVA, ovalbumin; TEC, thyroid epithelial cells; TGN38, trans-Golgi network protein-38.

Correspondence: Dr Colin Dayan, Division of Medicine, University of Bristol, Bristol Royal Infirmary, Bristol, BS2 8HW, UK.

© 2000 Blackwell Science Ltd

requirements of untransformed T cells) were used to detect the generation of class II–peptide complexes. The Fischer rat thyroid cell line, FRTL5, was used as these cells are not tumour-derived or virally transformed and retain many specialized functions of TEC (e.g. thyrotropin-dependent growth, iodine trapping and inducible MHC class II expression).[17]

MATERIALS AND METHODS

Reagents

Ovalbumin (OVA) and the control protein horse myoglobin (HMG) were obtained from Sigma (St Louis MO). Ninety-four overlapping 15-mer peptides spanning the entire OVA sequence and advancing by four amino acids at a time were custom synthesized by using multipin peptide synthesis technology (Chiron Mimotopes, Clayton, Victoria, Australia).

The following antibodies were used for cell immunofluorescence staining, and/or antigen-presentation blocking experiments: monoclonal murine anti-rat MHC class I (OX-18; Serotec) and MHC class II $RT1^B$ (OX-6; Serotec, Oxford, UK), mouse anti-rat trans-Golgi network protein-38 (TGN38) (2F7.1B1; Affinity BioReagents, Golden, CO), polyclonal rabbit antiovalbumin antibody (Sigma) and irrelevant mouse immunoglobulin (Serotec). Appropriate secondary fluorochrome-conjugated antibodies were purchased from Sigma or Pharmingen (Oxford, UK). Rat IFN-γ was a kind gift from P. H. van der Meide (TNO Primate Centre, Rijswijk, the Netherlands).

Cell lines

FRTL5 cells (donated by Dr L. Kohn and Dr N. Marshall) were cultured in 2.1.1 growth medium composed of Dulbecco's modified Eagle's minimal essential medium (DMEM; Gibco, Paisley, UK), Ham's F12 (ICN Flow, Basingstoke, UK) and MCDB 104 (Gibco), mixed in a 2 : 1 : 1 ratio, respectively. Medium was supplemented with 5 mU/ml of bovine thyrotrophin (Sigma), 5 μg/ml of bovine transferrin, 10 μg/ml bovine insulin, 10^{-8} M hydrocortisone, 4·5 μg/ml of ascorbic acid, 100 U/ml of penicillin, 0·1 mg/ml of streptomycin, 1 mM sodium pyruvate, 2 mM glutamine and 5% (v/v) heat-inactivated donor calf serum.

The murine interleukin-2 (IL-2) indicator cell line CTLL-2 (donated by Dr K. Hawrylowicz) was maintained in complete RPMI-1640 (Gibco), supplemented with antibiotics, 5×10^{-5} M 2-mercaptoethanolamine and 10% (v/v) heat-inactivated fetal calf serum (FCS; Gibco) to which 20 ng/ml of recombinant human IL-2 was added (Biogen, Geneva, Switzerland). The murine lymphoma hybridoma fusion partner BW5147 (clone BW5147.G.1.4 purchased from ECACC, Porton Down, Salisbury, UK) and human kidney cell line HEK-293 were both maintained in complete RPMI-1640 medium.

Animals and immunizations

All animal work was performed in accordance with local ethical guidelines. Six-week-old female Lewis rats were immunized at the tail base with 100 μg of OVA in 50 μl of complete Freund's adjuvant. The animals were killed after 10 days and inguinal

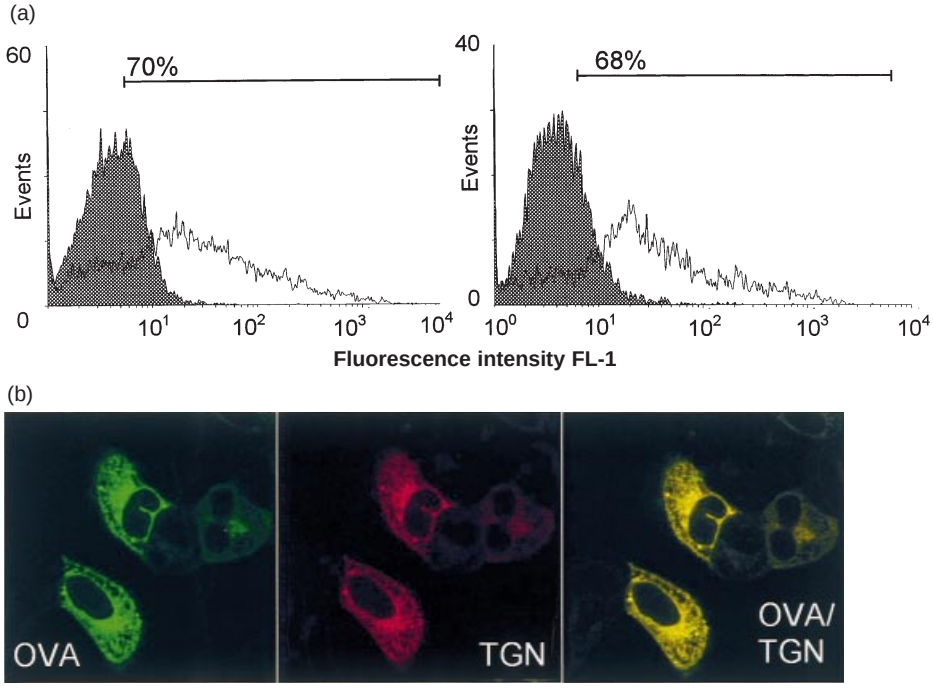

Figure 1. (a) Flow cytometry analysis of ovalbumin (OVA) expression by Fischer rat thyroid cell line (FRLT5) cells transfected with AdOVA–TGN38' in the absence (left panel) or presence (right panel) of 100 IU/ml of interferon-γ (IFN-γ). Shaded peak, control mouse immunoglobulin; open peak, specific anti-OVA staining. (b) Confocal microscopy demonstrating immunofluorescence staining for OVA (green), trans-Golgi network protein-38 (TGN38) (red) or co-localization expression (OVA/TGN) (yellow) in FRTL5 cells after transfection with AdOVA–TGN38'. Cells stained with secondary antibody alone were not visible on the settings used for this image.

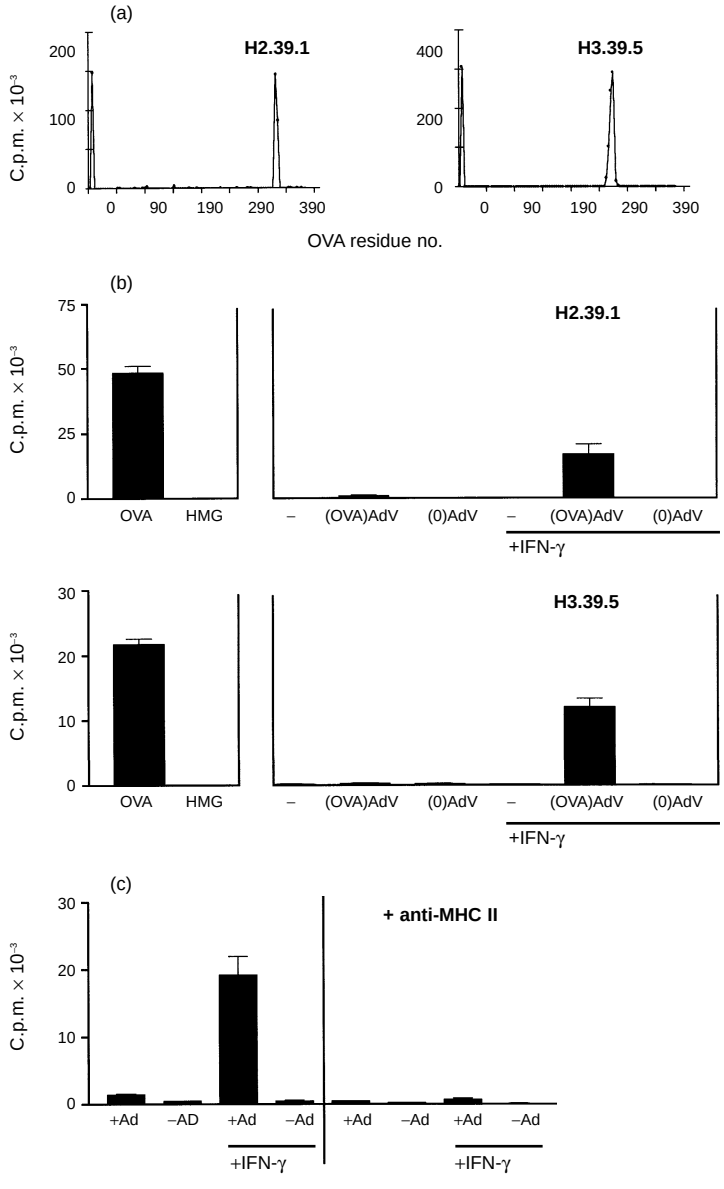

Figure 2. (a) Recognition of ovalbumin (OVA) (initial peak) and 15-mer overlapping peptides of OVA by hybridomas H2.39.1 and H3.39.5. (b) Right panels: response of H2.39.1 (upper panel, OVA 321–339 specific) and H3.39.5 (lower panel, OVA 261–279 specific) to endogenous OVA presented by Fischer rat thyroid cell line (FRLT5) cells in the presence or absence of 100 IU/ml of interferon-γ (IFN-γ). (–), untransfected; (OVA)AdV, transfected with AdOVA–TGN38′; (0)AdV, transfected with control virus Ad0. Left panel: responses of the same hybridomas to exogenous OVA or horse myoglobin (HMG) (100 μg/ml), presented by thymocytes, for comparison. (c) Presentation of OVA to H3.39.5 by FRTL5 cells transfected with AdOVA–TGN38′ (+Ad) or not transfected (– Ad) is blocked in the presence of 5 μg/ml of anti-major histocompatibility complex (MHC) class II antibody (OX-6; aMHC II). Similar results were seen with OVA 321–339 specfic hybridomas (data not shown). c.p.m., counts per minute.

lymph nodes were removed, disaggregated, pooled and used as a source of OVA-primed lymph node cells (LNC).

T-cell hybridomas

OVA-primed LNC were cultured for 3 days with 10 μg/ml of OVA in complete RPMI before fusing with BW5147 cells according to a standard polyethylene glycol-mediated cell-fusion technique.[18] Cells (hybridomas) able to survive in hypoxanthine/aminopterin/thymidine (HAT) selection medium were expanded in number, maintained in complete RPMI and subcloned between one and three times by limiting dilution. To assay for antigen reactivity, 1×10^5/well hybridoma cells were cultured in complete medium with antigen and 2×10^6 irradiated Lewis rat thymocytes in 96-well flat-bottomed plates (Nunc, Paisley, UK). Plates were then incubated for 48 hr and frozen at -20°. IL-2 release into the supernatant, as a measure of hybridoma activation, was then measured by using a CTLL-2 bioassay. One-hundred microlitre aliquots of defrosted supernatant were added to 100 μl of complete RPMI-1640 containing 2×10^4 CTLL-2 cells that had been starved of IL-2 for 18 hr. Cultures were then incubated for 48 hr, pulsed with 0·5 μCi of [^{3}H]deoxythymidine, harvested onto filtermats and [^{3}H] uptake

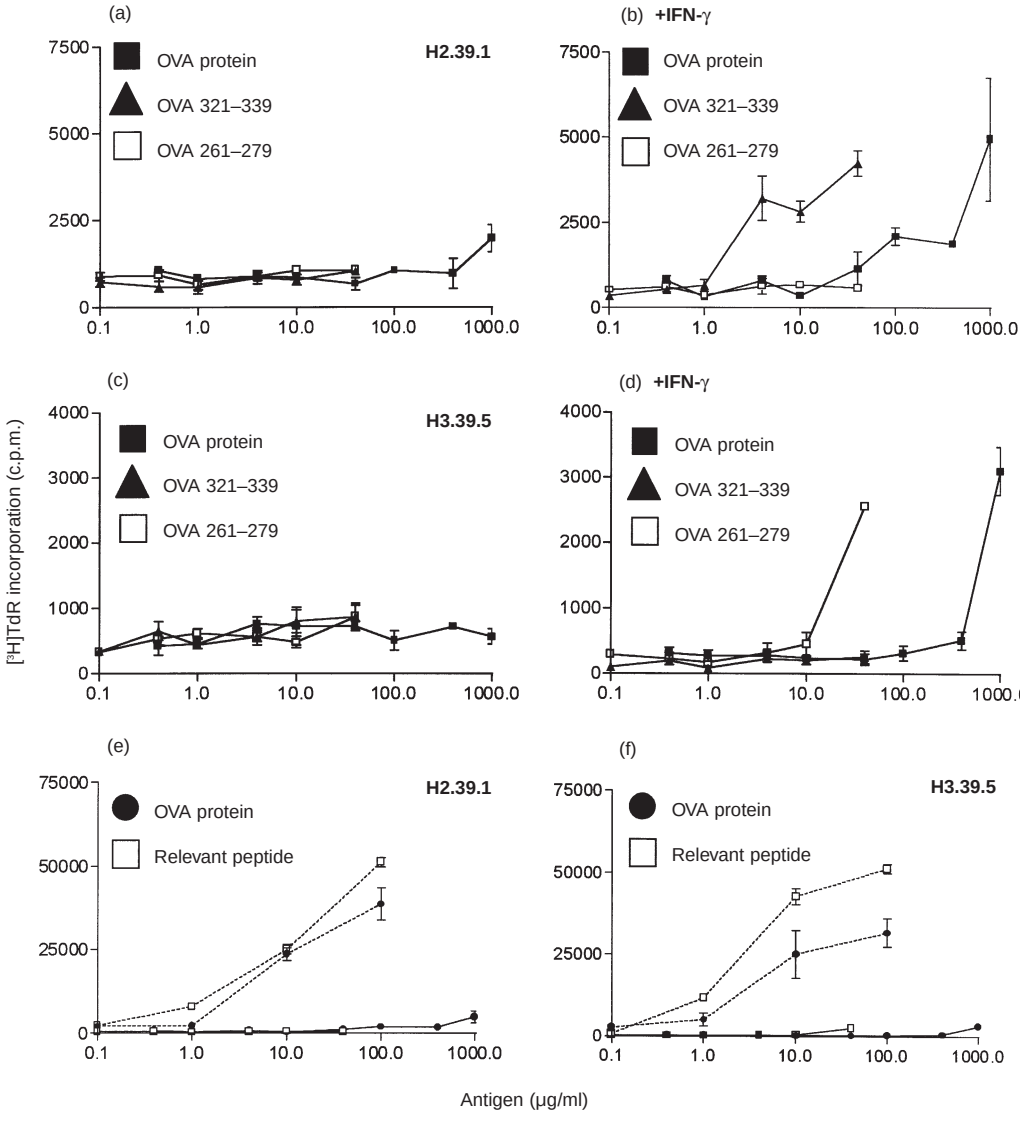

Figure 3. Fischer rat thyroid cell line (FRLT5) cells are ineffective at presenting exogenous antigen. (a–d): responses of H2.39.1 (a, b) and H3.39.5 (c, d) T-cell hybridoma clones to exogenous ovalbumin (OVA), OVA 321–339 and OVA 261–279 peptides, presented by FRTL5 cells after no exposure (a, c) or exposure (b, d) to 100 IU/ml of interferon-γ (IFN-γ). Small, yet specific, responses are seen. By comparison, the responses of H2.39.1 (e) and H3.39.5 (f) clones to exogenous OVA or relevant peptide are much greater and occur at lower antigen concentration when presented by professional antigen-presenting cells (APC) such as thymocytes (dashed line) as compared to FRTL5 cells (solid line). Note the difference in *y*-axis scale. c.p.m., counts per minute.

was measured by scintillation counting (Wallac, Turku, Finland). Results are expressed as mean counts per minute (c.p.m.) ± standard error of the mean (SEM) of triplicate CTLL-2 cultures.

For antigen-presentation studies, FRTL5 cells at 70–80% confluence were precultured for 48 hr with 100 IU/ml of recombinant rat IFN-γ (to induce MHC class II expression) and replication-deficient AdOVA–TGN38' to induce OVA expression (see below) in 96-well flat-bottomed wells. Cells were then washed twice *in situ* to remove residual viral particles, and 1×10^5 T-cell hybridoma cells were added in 100 μl of 2 : 1 : 1 medium for a further 48 hr before freezing and assaying using the CTLL-2 bioassay, as described above. For MHC-blocking experiments, antibodies were added together with the hybridoma cells.

Generation of AdOVA–TGN38' adenovirus

The pcDNAI(OVA) plasmid encoding full-length OVA was kindly provided by Dr N. Shastri (University of California, Berkeley, CA). A 1·28-kbp section of OVA, corresponding to amino acid residues 26–407 of whole OVA plus flanking in-frame *Bcl*I restriction sites, was amplified by using the polymerase chain reaction (PCR) and ligated into the *Bcl*I restriction site of the pMEP4(TGN38') construct, which contains the full-length rat cDNA for the type I integral membrane protein, TGN38.[19,20] The' indicates the S331A mutation introduced into the cytosolic domain of TGN38, which promotes cell-surface expression.[20] The resultant TGN38'–OVA insert therefore codes for a chimaeric protein that targets OVA to the trans-Golgi and cell membranes. The TGN38'–OVA sequence was cloned into pXCXCMV to

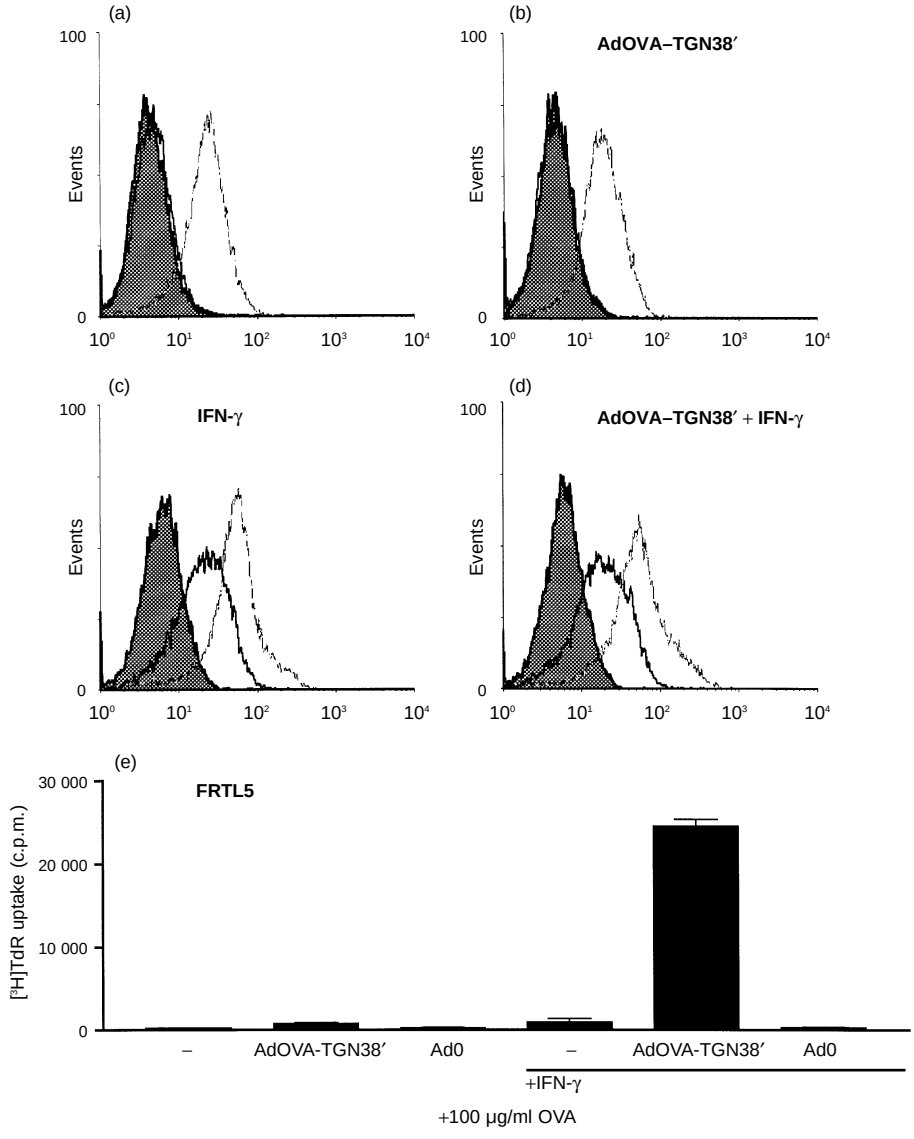

Figure 4. Flow cytometry analysis of Fischer rat thyroid cell line (FRLT5) cells (a, c) or FRTL5 cells transfected with AdOVA–TGN38' (b, d) in the absence (a, b) or presence (c, d) of 100 IU/ml of interferon-γ (IFN-γ) to show lack of effect on major histocompatibility complex (MHC) expression. Shaded peak, irrelevant primary antibody; solid line, MHC class II; dashed line, MHC class I. Panel (e) indicates that addition of irrelevant Ad0 virus does not enhance the presentation of 100 μg/ml of exogenous ovalbumin (OVA) protein by FRTL5 cells to the T-cell hybridoma clone H3.39.5. Presentation by FRTL5 cells transfected with AdOVA–TGN38' is shown for comparison. c.p.m., counts per minute.

generate the adenoviral cloning vector pXCXCMV–TGN38'/OVA. A recombinant, E1-gene deleted replication-deficient AdOVA-TGN38' adenovirus was then generated by cotransfection of HEK-293 cells with pXCXCMV–TGN38'/OVA and helper plasmid pJM17, as previously described.[21,22] AdOVA–TGN38' adenovirus was then expanded by further growth in HEK-293 cells and purified by ultracentrifugation.[22] Aliquots of adenovirus were stored at −80°. An irrelevant control adenovirus (Ad0) with no functional insert was generated by cotransfection of HEK-293 cells with pXCXCMV and helper plasmid pJM17.

Immunofluorescence staining for confocal microscopy and flow cytometry

FRTL5 cells cultured on glass coverslips were incubated with purified virus for 36 hr in 2.1.1 medium. Cells were then washed, fixed by addition of neat ice-cold methanol and labelled with specific primary antibody for 30 min. After washing, cells were stained in the same manner with relevant fluorochrome-labelled second-layer antibodies and examined for immunofluorescence using a Lecia DM-IRBE upright epifluorescence microscope attached to a Leica TCS-NT confocal laser scanning system (Leica, Milton Keynes, UK). For flow cytometry, 2×10^5 detached FRTL5 cells were incubated in the presence of specific antibody in 50 μl of prechilled phosphate-buffered saline (PBS) containing 5% (v/v) FCS for 30 min at 4°, washed and then incubated with specific fluorochrome-labelled second-layer antibodies for 30 min at 4° before passing through the a cytometer (FACSCalibur; Becton Dickinson).

RESULTS

Presentation of endogenous OVA by FRTL5 cells

To investigate the ability of FRTL5 cells to present endogenous antigen, an adenoviral vector encoding a chimaeric OVA/TGN38' protein was constructed (see the Materials and methods) in which the N-terminus of the TGN protein, TGN38, provides an effective signal sequence for the fusion protein to enter the endoplasmic reticulum, and the mutated C-terminal cytosolic domain of TGN38' (S331A mutation) targets the protein to the TGN, the cell surface and the lysosomal membrane system.[19,20] In this manner, OVA/TGN38' mimics endogenous membrane-associated proteins, such as thyroid peroxidase and the thyroid-stimulating hormone receptor, which form the majority of self-antigens identified to date.[23] Use of an adenoviral expression system allows a high rate of transfection and expression in FRTL5 cells that is not achievable by using other transfection methods.[24]

Flow cytometric analysis of FRTL5 cells exposed to AdOVA/TGN38' adenovirus for 36 hr confirmed surface expression of OVA and that a very high proportion of cells were expressing recombinant viral protein at the cell surface – typically around 70% (Fig. 1a). In addition, the level of infection and expression was shown not to be affected by exposure to IFN-γ (Fig. 1a, right panel). Confocal imaging of transfected cells confirmed that, as expected,[22] OVA/TGN38' is present on intracellular membranes (apparently including those of the TGN, Fig. 1b) in addition to the cell surface.

The major T-cell epitopes of OVA in the Lewis rat (which shares MHC class II haplotype with the Fischer rat – $RT1^l$ but is easier to immunize) were then mapped using primed lymph node cells and 94 overlapping peptides spanning the OVA sequence (data not shown). Two dominant sequences were identified (OVA 261–279 and 321–339). A panel of cloned rat × mouse T-cell hybridomas with the ablility to recognize one or other of the epitopes as well as whole OVA was then generated, and class II restriction was confirmed using blocking anti-MHC class I (OX-18) and class II (OX-6) monoclonal antibodies (mAbs) (Fig. 2a and data not shown). Thymocytes were used as antigen-presenting cells (APC), common practice in rat immunology because they are more effective than unpurified splenocytes for stimulating T cells.[25]

OVA-specific hybridomas were then exposed to transfected FRTL5 cells expressing OVA–TGN38'. A high degree of T-cell hybridoma activation was observed (Fig. 2b). This response was dependent on prior IFN-γ exposure to induce MHC class II molecule expression on the FRTL5 cells (Fig. 2b) and was blocked by anti-MHC II antibodies (Fig. 2c), confirming that the hybridomas were indeed detecting OVA–MHC class II complexes on the cell surface. Prior infection of FRTL5 with irrelevant Ad0 adenovirus (possessing no recombinant insert) did not result in any T-cell hybridoma activation (Fig. 2b). Note that FRTL5 cells generated peptide–MHC complexes from endogenous OVA containing both of the major epitopes identified using lymph node cells (Fig. 2b). Previously, there has been concern that processing by non-professional APC would generate new 'cryptic' epitopes from self-antigens, thereby bypassing self tolerance mechanisms. We found no evidence of this in our system.

Presentation of exogenous OVA by FRTL5 cells

In contrast to endogenously expressed OVA, presentation of exogenous OVA by FRTL5 cells was found to be very inefficient. Some activation of OVA-specific T-cell hybridomas was seen and shown to be epitope specific and dependent on MHC class II induction with IFN-γ (Fig. 3a, 3b, 3c, 3d). However, concentrations of OVA 10–100-fold higher than those needed to activate the hybridomas with bone-marrow derived APC were required (Fig. 3e, 3f). Furthermore, the level of hybridoma activation by FRTL5 cells, even at the highest concentrations, was very much lower than that seen with either professional APC (Fig. 3e, 3f) or presentation of endogenous antigen by FRTL5 cells (compare Figs 3 and 2a, noting *y*-axis scale). Presentation of exogenously administered OVA peptides was also poor, although slightly more effective than presentation of whole OVA (Fig. 3). Taken together, these data suggest that exogenous antigen has limited access to the peptide loading compartment in FRTL5 cells. However, we cannot exclude that our peptides require further processing before binding to MHC class II molecules, in which case the inefficient presentation of exogenous antigen seen here may represent either a failure of antigen uptake or of processing of exogenously derived antigen.

Effects of adenoviral transfection on antigen presentation

Infection of thyroid cells with live reovirus has previously been shown to induce MHC class II expression.[26] Although our system used replication-defective adenoviral constructs in which only a limited number of adenoviral genes were expressed,[21] we were keen to exclude the possibility of a non-specific enhancing effect of the viral construct on antigen presentation by FRTL5 cells. Transfection with AdOVA/TGN38′ did not induce MHC class II expression by FRTL5 cells and did not enhance levels of class II expression after exposure to IFN-γ (Fig. 4a 4b, 4c, 4d). Furthermore, infection with adenovirus lacking the OVA insert was not found to correct defective presentation of exogenous antigen by FRTL5 cells (Fig. 4e). These experiments indicate that it is unlikely that the defective adenoviral construct used has similar effects as live reovirus on antigen processing and presentation by FRTL5 cells, although more subtle effects on the processing and peptide loading pathways cannot be completely excluded.

DISCUSSION

Our results provide the most direct evidence to date that endogenous self-antigens can access the inducible MHC class II pathway in specialized epithelia, such as thyroid cells. The potent activation of T-cell hybridomas, seen after presentation of endogenously expressed antigen by FRTL5 cells, demonstrates clearly for the first time that competent antigen processing pathways exist within TEC and that processed endogenous antigen is loaded onto MHC class II molecules successfully. Furthermore, these experiments also indicate that sufficient levels of MHC class II molecules are expressed at the cell surface, after IFN-γ induction, to activate T-cell hybridomas. By using a thyroid cell line, we excluded a contribution by any other APC in this process, and the use of T-cell hybridomas allowed us to study antigen processing and class II loading in

FRTL5 cells separately from the costimulatory potential of these cells. We studied the presentation of a membrane-associated test antigen that we believe mimics the cellular distribution of several well-characterized self-antigens which are known to be involved in the autoimmune process.[23] Access of antigens from other parts of the cell, notably the cytosol, to the MHC class II pathway was not examined.

Although we believe that we have used the 'cleanest' experimental system employed to date to study access of endogenous antigen to the class II pathway in thyroid cells, there remain several technical uncertainties. First, even though FRTL5 cells are non-virally transformed and express many differentiated functions, it is still possible that they are not truly representative of normal thyroid cells. Second, levels of the test endogenous antigen studied will probably be higher than that of many self-antigens and although we have attempted to mimic a typical cellular distribution of autoantigen, there may be important differences. Finally, it remains possible that the adenoviral expression system used is able to alter the antigen processing machinery in FRTL5 cells. However, such viral effects tend to impair, rather than enhance, antigen presentation[27] and we have excluded an enhancing effect similar to that seen with reovirus.[26]

Our finding that, in contrast to their ability to present endogenous antigen, FRTL5 cells are inefficient at presenting exogenous antigen may explain why several previous workers have had limited success in studies with exogenous antigen.[13,15] However, it contrasts with the finding that human TEC derived from autoimmune glands can effectively present exogenously supplied viral peptides to T cells.[6,8] This discrepancy may reflect contamination of human TEC preparations with small numbers of dendritic cells, a difference between human TEC and FRTL5 cells, or an enhanced presentational capacity of TEC involved in autoimmune disease.

Our demonstration that endogenous antigens can access the inducible class II pathway in thyroid cells has important implications for autoimmunity. It indicates that in the presence of factors able to induce MHC class II expression in thyroid cells, such as IFN-γ, self-antigen is no longer 'sequestered', but available to CD4 T cells in sufficient quantities to result in T-cell activation. Although TEC lack expression of B7.1 and B7.2,[28,29] they do express CD40,[30] which may promote T-cell responses. On the other hand, TEC also express Fas ligand (CD95L),[31] which might be expected to promote apoptosis of T cells.[5] The diversity of the self-antigen response reported in spontaneous autoimmunity[7] and the 'determinant spreading' from the antigen used to induce disease to other autoantigens seen in animal models of autoimmunity over time[32] both strongly argue that self-antigens are actively presented to the immune system in autoimmune disease. Whether this occurs via the inducible MHC class II pathway studied here, or whether this pathway serves to inhibit this process by delivering negative signals *in vivo*, remains to be determined.

ACKNOWLEDGMENTS

This work was funded by The Wellcome Trust (Grants 042892, 046451), the United Bristol Healthcare Trust, the Charing Cross Hospital Special Trustees (C. M. D.) and The Nuffield Foundation (C. M. D.). Confocal microscopy was made possible by a Medical Research Council Infrastructure Award (G4500006) made to the University of Bristol. Acknowledgments to Professor C. Linington, Dr S. Anderton, Dr C. Hawrylowicz, Dr Marian Ludgate and Professor S. L. Lightman for much advice and support.

REFERENCES

1. Dayan C.M. & Daniels G.H. (1996) Chronic autoimmune thyroiditis. *N Engl J Med* **335,** 99.
2. Hanafusa T., Pujol-borrell R., Chiovato R., Russell R.C.G., Doniach D. & Bottazzo G.F. (1983) Aberrant expression of HLA-DR antigen on thyrocytes in Graves' disease: relevance for autoimmunity. *Lancet* **ii,** 1111.
3. Todd I., Pujol-Borrell R., Hammond L.J., Bottazzo G.F. & Feldmann M. (1985) Interferon-gamma induces HLA-DR expression by thyroid epithelium. *Clin Exp Immunol* **61,** 265.
4. Bottazzo G.F., Pujol-Borrell R., Hanafusa T. & Feldmann M. (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* **ii,** 1118.
5. Dayan C.M., Elsegood K.A. & Maile R. (1997) FasL expression on epithelial cells: the Bottazzo–Feldmann hypothesis revisited. *Immunol Today* **18,** 203.
6. Londei M., Lamb J.R., Bottazzo G.F. & Feldmann M. (1984) Epithelial cells expressing aberrant MHC Class II determinants can present antigen to cloned human T cells. *Nature* **312,** 639.
7. Dayan C.M., Londei M., Corcoran A.E. *et al.* (1991) Autoantigen recognition by thyroid-infiltrating T-cells in Graves' disease. *Proc Natl Acad Sci USA* **88,** 7415.
8. Lombardi G., Arnold K., Uren J. *et al.* (1997) Antigen presentation by interferon gamma-treated thyroid epithelial cells inhibits interleukin-2 and supports interleukin-4 production by B7-dependent human T cells. *Eur J Immunol* **27,** 62.
9. Tandon N. & Weetman A. (1992) Thyroid cells in Graves' disease and Hashimoto thyroiditis stimulate allogeneic T-cells when pretreated with phorbol ester. *Clin Endocrinol* **37,** 274.
10. Eguchi K., Otsuba T., Kawabe Y. *et al.* (1988) Synergy in antigen presentation by thyroid epithelial cells and monocytes from patients with Graves' disease. *Clin Exp Immunol* **72,** 84.
11. Yeni P. & Charreire J. (1981) Syngeneic sensitization of murine lymphocytes on monolayers of thyroid epithelial cells. I. Study of proliferative response. *Cell Immunol* **62,** 313.
12. Hirose W., Lahat N., Platzer M., Schmitt S. & Davies T.F. (1988) Activation of MHC-restricted rat T cells by cloned syngeneic thyrocytes. *J Immunol* **141,** 1098.
13. Ebner S.A., Stein M.E., Minami M., Dorf M.E. & Stadecker M.J. (1987) Murine thyroid follicular epithelial cells can be induced to express Class II (Ia) gene products but fail to present antigen *in vitro*. *Cell Immunol* **104,** 154.
14. Czirjak L., Danko K., Gaulton G.N. & Stadecker M.J. (1988) Thyroid-derived epithelial cells acquire alloantigen presenting capabilities following X-irradiation and Class II antigen induction. *Eur J Immunol* **20,** 2597.
15. Sigillito R.J., Miller S., Tokuda N. & Levy R.B. (1989) Rat thyroid epithelial cells provide limited accessory function but fail to present allo-antigens *in vitro*. *Regional Immunol* **2,** 275.
16. Salamero J., Remy J.J. & Charreire J. (1987) Primary syngeneic sensitization on monolayers of thyroid epithelial cells. X. Inhibition of T cell proliferative responses by thyroglobulin-specific monoclonal antibodies. *Clin Immunol Immunopathol* **43,** 34.
17. Ambesi-Impiombato F.S., Parks L.A.M. & Coon H.G. (1980) Culture of hormone-dependent functional epithelial cells from rat thyroids. *Proc Natl Acad Sci USA* **77,** 3455.
18. Taniguchi M. & Miller J.F.A.P. (1978) Specific suppressive factors produced by hybridomas derived from the fusion of enriched suppressor T cells and a T lymphoma cell line. *J Exp Med* **148,** 373.

19. Girotti M. & Banting G. (1996) TGN38–Green Fluorescent Protein hybrid proteins expressed in stably transfected eukaryotic cells provide a tool for the real-time, *in vivo* study of membrane traffic pathways and suggest a possible role for rat TGN38. *J Cell Sci* **109,** 2915.
20. Roquemore E.P. & Banting G. (1998) Efficient trafficking of TGN38 from the endosome to the TGN requires a free hydroxyl group at position 331 in the cytosolic domain. *Mol Biol Cell* **9,** 2125.
21. Graham F.L. & Prevec L. (1995) Methods for construction of adenovirus vectors. *Mol Biotechnol* **3,** 207.
22. Geddes B.J., Harding T.C., Hughes D.S. *et al.* (1996) Persistent transgene expression in the hypothalamus following stereotaxic delivery of a recombinant adenovirus – suppression of the immune-response with cyclosporin. *Endocrinology* **137,** 5166.
23. Rapoport B., Portolano S. & McLachlan S.M. (1995) Combinatorial libraries – new insights into human organ-specific autoantibodies. *Immunol Today* **16,** 43.
24. Zeiger M.A., Takiyama Y., Bishop J.O., Ellison A.R., Saji M. & Levine M.A. (1996) Adenoviral infection of thyroid cells – a rationale for gene therapy for metastatic thyroid carcinoma. *Surgery* **120,** 921.
25. Sedgwick J.D., Macphee I.A.M. & Puklavec M. (1989) Isolation of encephalitogenic T cell clones in the rat – cloning methodology and interferon gamma secretion. *J Immunol Methods* **121,** 185.
26. Neufeld D.S., Platzer M. & Davies T.F. (1989) Reovirus induction of MHC Class II antigen in rat thyroid cells. *Endocrinology* **124,** 543.
27. Ploegh H.L. (1998) Viral strategies of immune evasion. *Science* **280,** 248.
28. Tandon N., Metcalfe R.A., Barnett D. & Weetman A.P. (1994) Expression of the costimulatory molecule B7/BB1 in autoimmune thyroid disease. *Quart J Med* **87,** 231.
29. Matsuoka N., Eguchi K., Kawakami A. *et al.* (1996) Lack of B7/BB1 and B7/B70 expression on thyrocytes of patients with Graves' disease. Delivery of costimulatory signals from bystander professional antigen-presenting cells. *J Clin Endocrinol Metab* **81,** 4137.
30. Faure G.C., Bensoussan-Lejzerowicz D., Bene M.C., Aubert V. & Leclere J. (1997) Coexpresion of CD40 and Class II antigen HLA-DR in Graves' disease thyroid epithelial cells. *Clin Immunol Immunopathol* **84,** 212.
31. Giordano C., Stassi G., Maria R.D. *et al.* (1997) Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* **275,** 960.
32. Lehmann P.V., Sercarz E.E., Forsthuber T., Dayan C.M. & Gammon G. (1993) Determinant spreading and the dynamics of the autoimmune T-cell repertoire. *Immunol Today* **14,** 203.